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Desiccation-inducible genes are related to N_2-fixing system under desiccation in a terrestrial cyanobacterium ☆

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ABSTRACT

Terrestrial cyanobacteria have various desiccation-tolerant systems, which are controlled by desiccation tolerance-related genes. *Anabaena* (*Nostoc*) sp. strain PCC 7120 is a derivative of the terrestrial cyanobacterium *Nostoc* and is a useful strain for molecular biological analysis. To identify desiccation tolerance-related genes, we selected and disrupted various genes (*all0801*, *all0875*, *alr3090*, *alr3800*, *all4052*, *all4477*, and *alr5182*) and examined their gene expression patterns and predicted their functions. Analyses of gene disruptants showed that viability of the disruptants only decreased under N_2-fixing conditions during desiccation, and the decrease in viability was negatively correlated with the gene expression pattern during desiccation. These data suggest that terrestrial cyanobacteria may acclimate to desiccation stress via N_2 fixation by using desiccation inducible genes, which are not only related to nitrogen fixation or nitrogen metabolism but also to other systems such as metabolism, transcription, and protein repair for protection against desiccation damage under nitrogen-fixing conditions. Further, a photosynthetic gene is required for desiccation tolerance. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

Cyanobacteria, the photosynthetic, O_2-evolving prokaryotes [1–3], grow in diverse habitats ranging from the tropics to the polar regions [4]. To survive in and acclimate to various conditions, some cyanobacteria have developed desiccation tolerance derived from their archaic origin. These cyanobacteria are subjected to repeated cycles of dehydration, desiccation, and rehydration and can survive under desiccated conditions for long periods. The desiccation-tolerant cyanobacterium *Nostoc commune* is a useful organism for physiological analysis [5–8]. However, the relationship between desiccation tolerance and photosynthesis has not been elucidated.

The genome sequence of the nitrogen-fixing filamentous cyanobacterium *Anabaena* (*Nostoc*) sp. PCC 7120 has been analyzed [9]. *Anabaena* is a useful organism for studying nitrogen fixation using molecular biology [10–13]. Previously, Katoh et al. [14,15] showed that *Anabaena* is a close relative of a terrestrial, desiccation-tolerant cyanobacterium, *Nostoc* sp. HK-01, and analyzed the transcriptome of *Anabaena* under desiccation. Higo et al. [16] analyzed desiccation-inducible gene disruptants and showed that the trehalose metabolism genes are related to desiccation tolerance. Comparing the gene expression patterns of *Anabaena* and *Nostoc* sp. HK-01 in response to desiccation, Yoshimura et al. [17] showed that the group 3 sigma factor gene (*sigJ*) regulates the synthesis of extracellular polysaccharide. Although the *Anabaena* transcriptome has been analyzed under conditions of desiccation [15,16], the functions of only a few desiccation-inducible genes have been elucidated.

Genomic analysis facilitates the investigation of gene function. However, the functions of several genes remain unclear because these genes may be expressed under special conditions, such as nitrogen fixation, heat, cold, osmosis, and salinity, or their functions may be complemented by other gene(s). Our previous study identified the nitrogen fixation-related gene *nifH2* as one of the desiccation-induced genes [15]. Given that *Anabaena* fixes nitrogen, desiccation-induced genes may be related to nitrogen fixation or nitrogen assimilation. However, the relationship between desiccation tolerance, and nitrogen fixation and assimilation has not been investigated.

In this study, the functions of desiccation-induced genes in *Anabaena* sp. PCC 7120 were analyzed using the gene disruption technique and an easy desiccation tolerance test in the presence or absence of a nitrogen source to investigate the relationship between desiccation tolerance and nitrogen metabolism (fixation and assimilation). Typical desiccation-induced genes described in Katoh et al. [15] were selected for analysis, along with a gene related to photosynthesis.

2. Materials and methods

2.1. Organism and culture conditions

Nitrogen-fixing *Anabaena* sp. PCC 7120 was grown at 28 °C in BG11 or BG11o medium (NO_3^--free for N_2-fixing condition) [18] with

Abbreviations: Chl, chlorophyll; PCR, polymerase chain reaction; PS II, photosystem II

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5 mM HEPES-NaOH (pH 7.5) at 20 to 40 μmol photons m^{-2} s^{-1} under continuous fluorescent illumination. To assess desiccation tolerance, cells were grown in 2.5 mL of BG11 or BG11o medium in a 12-well plate placed on a high-speed shaking table. Cell density was estimated by measuring optical density at 750 nm (OD_{750}) using a spectrophotometer (model UV-160A; Shimadzu, Kyoto, Japan; OD_{750} 1 = approximately 5×10^7 cells/mL). The concentration of chlorophyll extracted using methanol was measured at 665 nm (A_{665}) using a spectrophotometer and calculated with A_{665} 1 = 13.42 μg Chl/mL [19]. Wild-type *Anabaena* was maintained on BG11 solid medium supplemented with 1.2% (w/v) agar, 5 mM TES-KOH (pH 8.0), and 0.3% (w/v) sodium thiosulfate. *Anabaena* mutants were maintained on the same solid medium as wild-type cells in the presence of antibiotics (10 μg/mL spectinomycin [Sp] and 2 μg/mL streptomycin [Sm]), but were propagated in the absence of antibiotics for analytical experiments.

2.2. Construction of Anabaena gene disruptants

As described in Katoh et al. [8], the genes *all0801*, *all0875*, *alr3090*, *alr3800*, *all4052*, *all4477*, and *alr5182* were selected for gene-inactivated phenotype analysis. The functions of these genes were determined using BLAST homology search [20] and Pfam search and were compared with a large collection of multiple sequence alignments and hidden Markov models covering many common protein domains and families [21]. Gene names were designated using CyanoBase (Kazusa DNA Research Institute, http://bacteria.kazusa.or.jp/cyanobase/Anabaena/index.html). To inactivate the targeted genes, 1.2 to 1.3 kb of DNA was amplified with each set of forward and reverse primers containing an *Xho*I (underlined) site, as shown in Table 1. Each *Xho*I-digested PCR-amplified gene was ligated to the cloning vector pRL271. The SpR/SmR cassette was inserted into a selected restriction site in the middle of the PCR-amplified gene to disrupt the gene [22]. The constructed vector was transferred to *Anabaena* sp. PCC 7120 from *E. coli* using a conjugation method, as described previously [23]. The genes were inactivated by *sucB*-mediated positive selection for double recombination [22].

Gene inactivation was confirmed by PCR using the primers shown in Table 2.

2.3. Viability analysis of desiccated Anabaena

Wild-type and gene-disrupted *Anabaena* cells were grown in BG11 or BG11o medium up to the late logarithmic phase. The cells were harvested by centrifugation, and the pellets were suspended in fresh medium to a final volume corresponding to OD_{750} 10, based on the measured absorbance value before cell harvest. The concentrated cells (10 μL) were then spotted on a "mixed cellulose ester" filter paper (pore size, 0.45 μm; Toyo Roshi Co. Ltd., Tokyo, Japan), and desiccated in a Petri dish (90 × 15 mm) under continuous illumination with white fluorescent lamps (20 to 30 μmol photons m^{-2} s^{-1}) at 28 °C and 30% relative humidity for 0, 4, 8.5, and 24 h. In this experiment, the water content was continuously reduced from 100% to 0% in 8.5 h, as described in Katoh et al. [15] and Higo et al. [16]. The resuspended cell cultures were diluted with a chlorophyll base, and an aliquot of each cell culture was spotted on an agar plate containing growth medium supplemented with 0.6% sodium thiosulfate and 5 mM TES-KOH (pH 8.0). The plates were kept illuminated (20 to 30 μmol photons m^{-2} s^{-1}) at 28 °C for 3 days. Changes in viability were calculated based on microscopic determination of colony forming units, and the viability at 0 h was defined as 100%.

Table 1
Primers used for gene-disruption.

Gene name	Forward primer	Reverse primer
all0801	ACTCGAGGATTCGCAACTATCTC	ACTCGAGTATCTCTCGGTGCAAT
all0875	ACTCGAGATGTAACAGACCCTTA	ACTCGAGGTACATGATAACCAGC
alr3090	ACTCGAGTCTGAGTCTTCCTGGT	ACTCGAGCGTGGGAACGATGATA
alr3800	ACTCGAGATGCGGACGATGTTTA	ACTCGAGCCTACGGTAAGACTTC
all4052	ACTCGAGCGCAACAATTGCGAAT	ACTCGAGAGTACGGTACTATTCC
all4477	ACTCGAGGTATTGCCATTCCTAT	ACTCGAGGACAATTCATTTGCAT
alr5182	ACTCGAGCAAACATTACAACCAC	ACTCGAGCAGCAGTTTCAATTCC

Underline shows *Xho*I site.

Table 2
Primers used for segregation analysis.

Gene name	Forward primer	Reverse primer
all0801	GCAAGTGCAGCAGATTCA	TTTCCCCAAATCCGGGTA
all0875	GAAGACGGTACGTATCAA	CCATCATCACCAGCTTCA
alr3090	GGTGGTATTCTCTCCACA	ATATTCTAGCTGGAGCGT
alr3800	AAGGCAGCGCCTTCTAGA	CTTGCTAGGTACATAGAG
all4052	CTACACCAATGGCATGAA	GGAGATCGCTCATCTGTT
all4477	AGAACGTGCCAAGGTTGA	GCTTGCAGCATCCGAATT
alr5182	GATCTGTCGATCCAAGGT	CACCATAGCCACATTGGT

2.4. Microscopic analysis of Anabaena cells

Wild-type *Anabaena* and disruptants were observed under an optical microscope. To observe chlorophyll fluorescence, excitation light at 435 nm was used.

2.5. Measurement of intracellular trehalose and sucrose

Desiccation was carried out as described in Section 2.4. Modifications were as follows. One hundred microliters of cell suspension was spotted on a filter paper and desiccated. Desiccated cells and filters were soaked in 80% ethanol and treated at 65 °C for 3 h. The supernatant was recovered and evaporated to dryness. Samples were dissolved in a small amount of water and treated with trehalase or invertase to determine the trehalose or sucrose content, respectively. The product glucose was enzymatically measured using the Glucose CII-test (Wako Pure Chemicals, Osaka; [16]) and used for estimating the trehalose and sucrose content.

3. Results

3.1. Selected genes and categories

The selected genes and categories are summarized in Table 3 and Fig. E1. The gene expression categories described in Katoh et al. [15] were rearranged as follows: (i) type U1 included immediate upregulated genes, (ii) type U2 included immediate upregulated genes that were downregulated after the middle phase of desiccation, (iii) type U3 included genes upregulated during the middle phase of desiccation, and (iv) type U4 included genes upregulated after the middle phase of

Table 3
Selected genes and their predicted functions.

Gene name	Predicted function	Gene expression pattern
all0801	Photosystem II-associated peripheral protein	–
all0875	Putative alpha-glucanotransferase with an alpha-amylase catalytic domain	U2
alr3090	Hypothetical protein slightly homologous to manganese catalase	U1
alr3800	SigB2 (*sigB2*) and group 2 sigma 70-type sigma factor E, SigE (*sigE*)	U3
all4052	Transketolase, may participate in sugar metabolism	U1
all4477	DnaK-type molecular chaperone DnaK (*dnaK*)	U4
alr5182	Oxidoreductase, which is also homologous to dehydrogenase	U4

desiccation. The selected genes were classified according to their gene expression patterns as follows: type U1, *alr3090* and *all4052*; type U2, *all0875*; type U3, *alr3800*; and type U4, *all4477* and *alr5182*. Only *all0801* was not classified because it was not upregulated.

3.2. Predicted functions of selected genes

Target genes were selected based on their expression pattern described by Katoh et al. [15], and their functions were predicted using BLAST homology search and Pfam search (Table 3). The predicted function of each gene was as follows: *all0801* encodes a photosystem II-associated peripheral protein; *all0875*, a putative alpha-glucanotransferase with an alpha-amylase catalytic domain, which is induced under low temperature conditions [24]; *alr3090*, a hypothetical protein that is slightly homologous to manganese catalase; *alr3800*, which has 2 names, *sigB2* and group 2 sigma 70-type sigma factor E (*sigE*), but belongs to the *sigB* group according to phylogenetic analysis [17]; *all4052*, a transketolase, which may participate in sugar metabolism; *all4477*, a DnaK-type molecular chaperone DnaK (*dnaK*), which is weakly homologous to the other *dnaK* genes in *Anabaena*; and *alr5182*, an oxidoreductase, which is also homologous to dehydrogenase. The selected genes are associated with many systems, such as energy synthesis, cellular maintenance, and cellular protection.

3.3. Desiccation tolerance test

Although the desiccation-induced gene disruptants exhibited declining viability, the decline was observed in nitrogen-free condition. Fig. 1 shows the colony forming ability (viability) of wild-type *Anabaena* and the gene disruptants after desiccation stress. In nitrogen-rich condition, no significant difference was observed between the wild types and disruptants, except in the case of the photosynthesis-related *all0801* gene (Fig. 1A). On the other hand, all the gene disruptants exhibited declining viability in nitrogen-free condition (Fig. 1B). According to Katoh et al. [15], these desiccation-induced genes were classified into 4 expression patterns (See Section 3.1. Selected genes and categories, and Fig. E1). Disruption of the desiccation-induced genes *alr3090* and *all4052* (type U1) elicited a rapid loss of viability within 4 h of desiccation. A similar result was obtained with the *all0875* disruptant (type U2). Compared to types U1 and U2, the middle phase type U3 gene *alr3800* disruptant exhibited a gradually declining viability. Analysis of the *alr3800* disruptant revealed that the decrease in viability of the type U3 gene disruptant was slower than that of type U1 and U2 and faster than that of type U4. The observation of the gene disruptant was correlated and overlapped with the gene expression pattern, i.e., gene disruption influenced desiccation tolerance. Disruptant of the constitutively expressed *all0801* also showed declining viability during desiccation irrespective of the nitrogen condition.

3.4. Effects of osmoprotectants in drought tolerance

Osmoprotectants are known to protect intracellular conditions; a previous study has reported a relationship between osmoprotectants and desiccation tolerance [16]. *Anabaena* produces sucrose and trehalose [16,25] as osmoprotectants, thereby acquiring desiccation tolerance. Fig. 2 shows the relationship between desiccation time and intracellular osmoprotectant content. The extent of osmoprotectant accumulation in the presence or absence of nitrogenous nutrient was not significantly different between wild-type cells and gene disruptants.

3.5. Cell growth and chlorophyll analysis

To analyze the effects of gene disruption, growth curves of wild-type *Anabaena* and gene disruptants were measured (Fig. 3). In nitrogenous nutrient-containing medium, only the *all0801* disruptant exhibited growth inhibition (Fig. 3A). In nitrogen-free medium, the *all0875*, *alr3800*, and *all4052* disruptants exhibited growth inhibition (Fig. 3B). Comparison of the chlorophyll concentration between nitrogenous nutrient-free medium and nitrogen-rich medium revealed that the chlorophyll concentration per OD_{750} (corresponding to cell number) (Chl/OD_{750}) of cells grown under nitrogen-free condition was 23 to 49% lower than that of cells grown under nitrogen-rich condition (Table 4). In general, the chlorophyll content of most desiccation-induced gene disruptants was 9 to 27% lower than that of wild-type cells. In contrast, the chlorophyll content of the *all0801* disruptant was 9% higher than that of wild-type cells (Table 4).

3.6. Microscopic analysis of cellular features

To analyze the effects of gene disruption, cellular features of wild-type *Anabaena* and gene disruptants were examined (Fig. 4). In nitrogen-containing medium, only the *all0801* disruptant exhibited a reduction in cell size (Fig. 4). In nitrogen-free medium, the *all0875* and *all4052* disruptants exhibited fragmentation, whereas the *alr3800* disruptant exhibited aggregation (Fig. 4B).

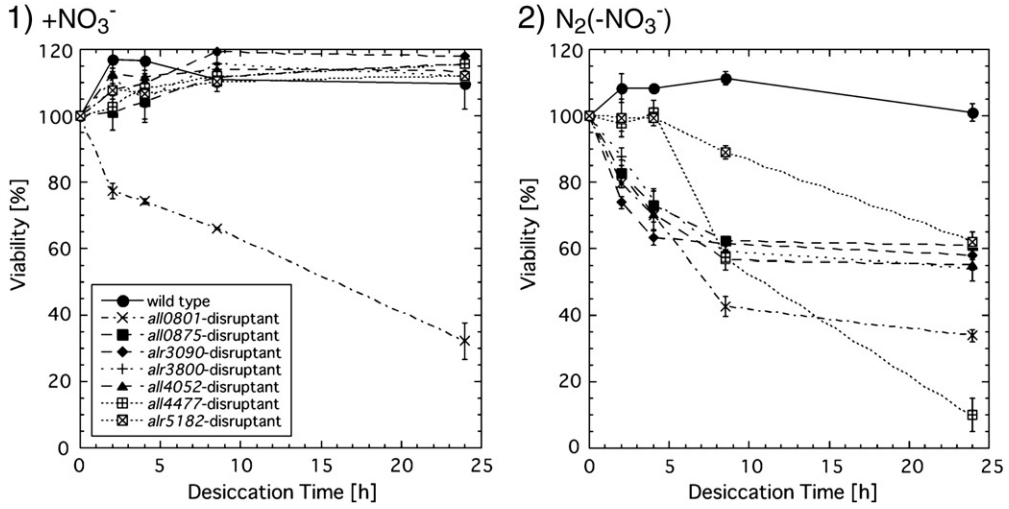

Fig. 1. Colony forming abilities of wild-type *Anabaena* and gene disruptants during desiccation stress. Mean viabilities and standard deviations were calculated from at least 3 independent spots using the same concentration of cells. Symbols are shown in the figure. 1) Nitrate-containing condition. 2) Nitrate-free condition.

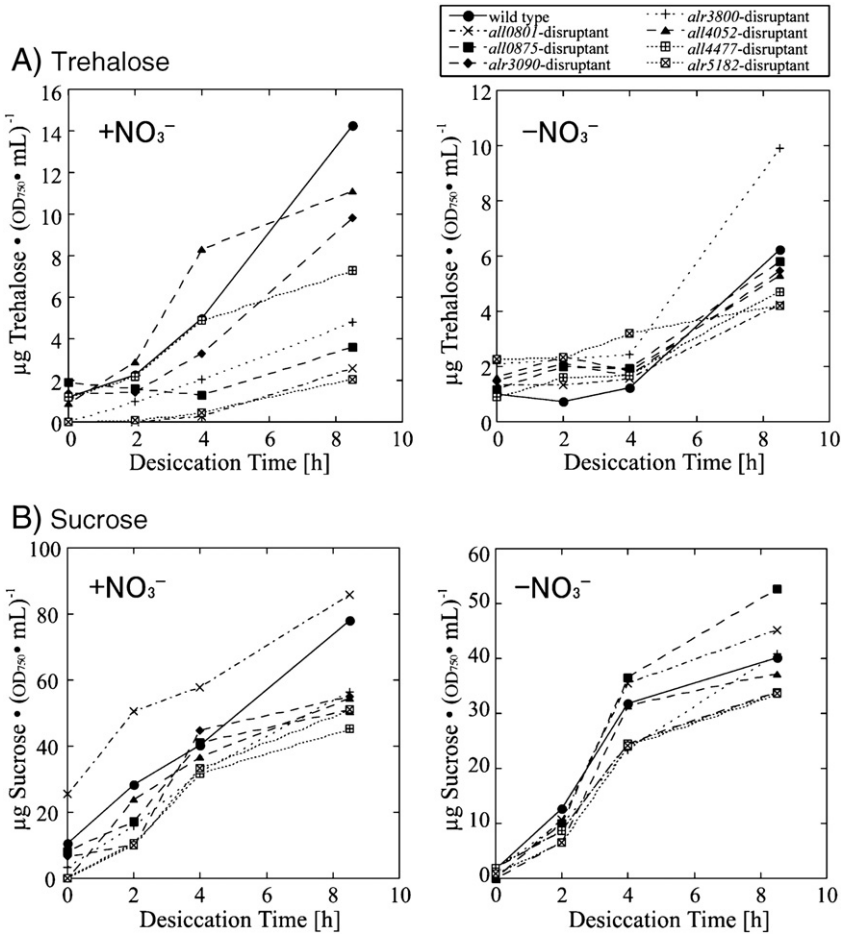

Fig. 2. Trehalose (A) and sucrose (B) accumulation in wild-type *Anabaena* and gene disruptants during desiccation stress. Symbols are shown in the figure. 1) Nitrate-containing condition. 2) Nitrate-free condition. Unit of vertical axis line indicates sugar concentration per OD_{750} corresponding to cell number.

4. Discussion

In this study, desiccation-induced gene disruptants exhibited desiccation sensitivity under nitrogen-free condition, suggesting that the desiccation-induced genes are related to nitrogen fixation or nitrogen metabolism. Genes related to various stresses, such as heat, low temperature (*all0875*, homologous to *lti2*) [24], osmotic pressure and salinity (*all0875*) [26], are induced in response to desiccation and may protect the nitrogen fixation system from various stresses. Microscopic analysis suggests that some disruptants exhibit cell fragmentation or aggregation, and growth inhibition under nitrogen-free condition, implying that some of the genes are related to the formation of nitrogen-fixing cells (heterocyst) and are linked to vegetative cells.

In desiccation inducible genes, the correlation between decreasing viability of the gene disruptant and the gene expression pattern under desiccation suggests that the desiccation inducible genes may function under desiccation condition.

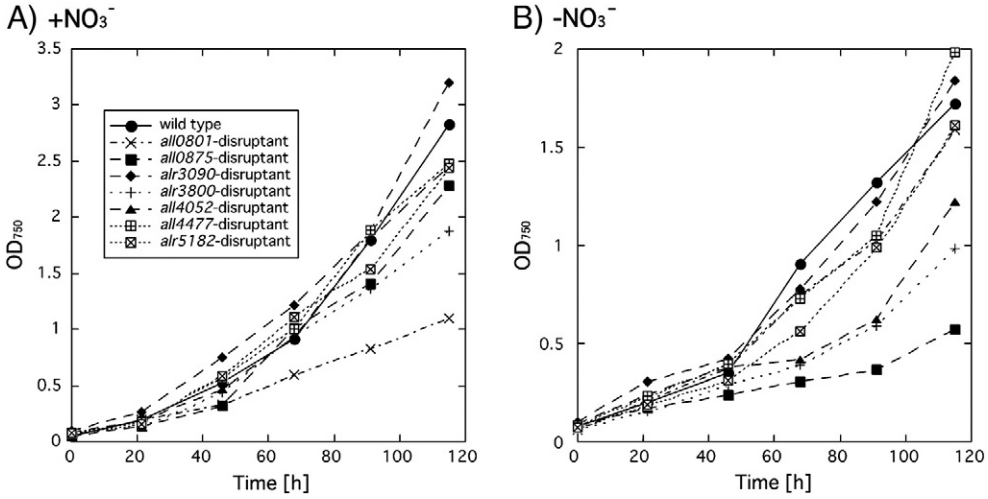

Fig. 3. Growth curves of wild-type *Anabaena* and gene disruptants. Symbols are shown in the figure. 1) Nitrate-containing condition. 2) Nitrate-free condition.

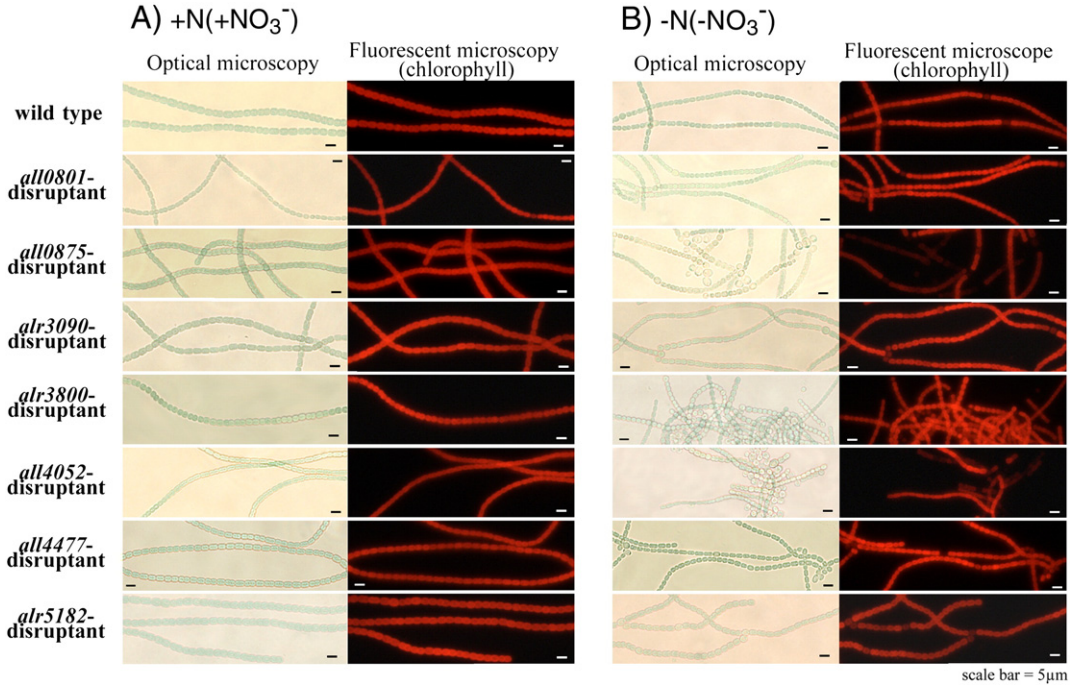

Fig. 4. Microscopic analysis of wild-type *Anabaena* and gene disruptants grown under 1) nitrate-containing and 2) nitrate-free conditions.

The desiccation tolerance test strongly indicates that the early-induced genes function during the early phase of desiccation. The phenotypes of the *all0875*, *alr3090*, and *all4052* disruptants imply that the U1 and U2 category genes are associated with sugar metabolism and radical scavenging. To acclimate during the early phase of desiccation, cells may protect against intra- and extracellular oxidative damage via various metabolic pathways, including energy synthesis. Metabolism of sucrose and trehalose is also activated to protect cells from desiccation damage, as discussed by Higo et al. [16].

Compared to the U1 and U2 gene disruptants, the middle phase type U3 gene *alr3800* disruptant exhibited gradually declining viability. Analysis of the *alr3800* disruptant revealed that the decrease in viability of the type U3 gene disruptant was slower than that of types U1 and U2 and faster than that of type U4.

The sigma factor B2 gene (*sigB2*, highly homologous to *alr3800*) product is associated with the induction of heat shock protein [27]. Recently, Osanai et al. [28] suggested that group 2 sigma factor SigE

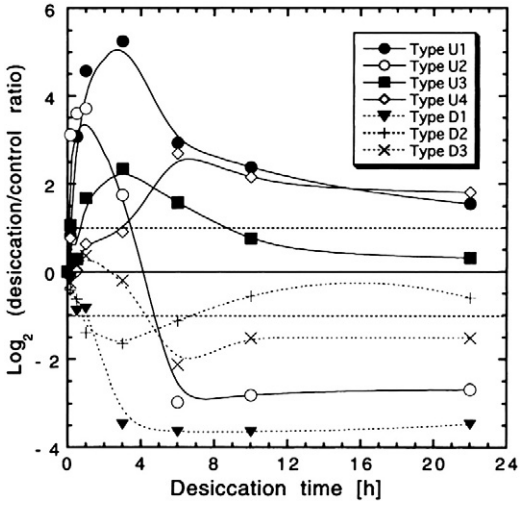

Fig. E1. Time courses of typical gene expression of several genes in *Anabaena* PCC 7120 during desiccation.
From [15].

controls the expression of sugar catabolic genes. Considering that the *alr3800* gene is expressed in both vegetative cells and nitrogen-fixing heterocysts [29], it may prepare the cell for heterocyst differentiation and protect against various stresses by regulating the expression of other genes, including those related to energy synthesis and protein repair, under nitrogen-fixing condition. Yoshimura et al. [17] reported that the sigma factor SigJ is a regulator of extracellular polysaccharide, which is a desiccation tolerant material, and is expressed during the middle phase of desiccation in *Nostoc* sp. This category includes transcription factors [15], suggesting that the middle phase genes are not only efficient in the middle phase of desiccation but also in the late phase of desiccation.

The late phase type U4 gene disruptants *all4477* and *alr5182* exhibited the slowest decrease in viability compared to the U1, U2, and U3 gene disruptants. The viability patterns of the *all4477* and *alr5182* disruptants were consistent with their gene expression patterns, suggesting that the late phase genes are efficient during the late phase of desiccation. The U4 category includes the heat shock protein and oxidoreductase genes. *Anabaena* has 5 *dnaK* genes, and the *all4477* product shares weak homology with them, suggesting that this gene may have a specific function in desiccation tolerance. The DnaK-type chaperone not only repairs denatured proteins using ATP but also degrades denatured or aggregated proteins in cooperation with the ClpB protein [30]. The *Anabaena clpB* genes were induced in the late phase of desiccation [15,16]. Using the energy from sugar metabolism in the early phase of desiccation, the ATP-driven DnaK system may repair denatured proteins related to housekeeping, and degrade unnecessary or irreparably denatured proteins. This protein repair and degradation system may be necessary for desiccation tolerance. It is also required for maintaining intracellular redox conditions. These data suggest that oxidoreductase may function during severe water deficiency and control redox conditions. The type U4 genes may function to protect against protein denaturation (by repairing or degrading irreparable proteins) and maintain intracellular conditions during low-water situations.

Gene disruption analyses of various unknown genes suggest that some of the desiccation-induced genes may function in vegetative cells and heterocysts under nitrogen-fixing conditions, since desiccation-induced genes have been investigated under nitrogen-rich conditions [15] and the *alr3800* (sigma factor) gene is expressed in both

Table 4
Chlorophyll content in the cells under nitrogenous nutrient-free and nitrogenous nutrient-rich conditions.

	wild-type	*all0801-disruptant*	*all0875-disruptant*	*alr3090-disruptant*	*alr3800-disruptant*	*all4052-disruptant*	*all4477-disruptant*	*alr5182-disruptant*
$-NO_3/+NO_3$	70.9	77.3	56.5	55.4	64.6	56.5	61	51.7
(%, mean [SD])	(2.1)	(5.2)	(3.3)	(4.2)	(2.6)	(7.6)	(4.2)	(2.6)
Relative to wild-type (%)	100	109.1	79.8	78.1	91.1	79.8	86.1	72.9

SD.: standard deviation.

vegetative cells and heterocysts [29]. Considering the localization of the gene products, desiccation-induced genes may function not only in nitrogen fixation and assimilation but also in heterocyst formation. Viewed from a different angle, to protect from desiccation damage, metabolism-related genes, such as the *all0875* gene encoding alpha-glucanotransferase, trehalose metabolism genes [15,16], and the *alr5182* gene (homologous to short-chain aldehyde dehydrogenase/reductase or oxidoreductase), are expressed under desiccation condition. This suggests that metabolic systems are also related to protection from desiccation damage. Given that trehalose metabolism, a sugar metabolic system, synthesizes not only trehalose but also glucose, trehalose metabolism genes may be upregulated during desiccation to synthesize desiccation protectant energy synthesis cycle substrate, and extracellular polysaccharide. Furthermore, to protect from desiccation damage, the *all4477* (chaperone) gene may function as a protein repair or degradation system and the *all3090* (homologous to Mn-catalase) gene may function as an antioxidant enzyme. Each metabolic and protection system may be related to desiccation tolerance, and the phenotype may appear under nitrogen-fixing condition. This suggests that the cellular status of *Anabaena* is strictly controlled by gene expression under nitrogen-fixing conditions, and this severe control cannot be optimized without desiccation-inducible genes under low-nitrogenous nutrient and desiccation conditions.

Although the desiccation-induced gene disruptants were sensitive to desiccation, some of the disruptants were unable to grow under nitrogen-free condition. Analysis of the chlorophyll/cell ratio and growth rates suggests that the desiccation-induced gene products are related to chlorophyll synthesis associated with nitrogen fixation. The *all0875*, *alr3800*, and *all4052* disruptants exhibited lower growth rates and cell aggregation or fragmentation under nitrogenous nutrient-free condition. These genes encode a putative alpha-glucanotransferase, sigma factor, and transketolase, respectively. The results suggest that these genes may play a role to enhance the cell membrane, cell wall, or extracellular matrix under nitrogenous nutrient-free condition.

In contrast, disruption of the photosynthetic gene *all0801* suggests desiccation sensitivity irrespective of the presence of nitrogenous nutrients. This seems to agree with the fact that the *all0801* gene is not induced by desiccation but is constitutively expressed [15,16]. The product Psb28 is loosely associated with PSII [31]. The function of Psb28 is still unknown, but its structure and location have been reported [32,33]. However, the results suggest that Psb28 plays an important role not only in optimal PSII functioning but also in maintaining desiccation tolerance in *Anabaena*.

This study suggests that this desiccation test is a useful method for screening desiccation tolerance-related genes because the viability of gene disruptants is associated with the induction phase of desiccation tolerance-related genes. To validate this system, we analyzed these desiccation tolerance related genes using other experiments. Analyses of gene disruptants show that this system is efficient to observe the induction phase of desiccation tolerance-related genes. This system in conjunction with chlorophyll fluorescence may enable the identification of new photosynthetic genes associated with desiccation tolerance [34]. Thus, this system is useful for analyzing dehydration and rehydration of terrestrial cyanobacteria.

5. Conclusions

In this study, desiccation-induced gene disruptants exhibited desiccation sensitivity under nitrogenous nutrient-free condition, suggesting that the desiccation-induced genes may be related to not only nitrogen fixation or nitrogen metabolism but also other systems, such as metabolism, transcription, and protein repair, to protect against desiccation damage under nitrogen-fixing condition. The data further suggest that the expression patterns of the desiccation-induced genes are correlated with their functions. Furthermore, not only desiccation-induced genes but also constitutively expressed genes, such as a photosynthetic gene, are required for desiccation tolerance. In the future, this system may help to elucidate the mechanism of desiccation tolerance and may further contribute to functional analysis.

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